View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

fluorescence microscopy (TIRFM) is used to monitor the activity of individual fluorescently-labeled cellulases interacting with insoluble cellulose substrates. Using wide field imaging, we are able to simultaneously record the motion of multiple, individual cellulases with nanometer spatial resolution. Time-resolved localization microscopy provides insights on: (i) binding and diffusion of enzyme to 'active' sites on cellulose; (ii) lifetime of enzyme activity for hydrolysis; (iii) enzyme processivity; and (iv) the nature of synergy between cellulase enzymes.

1378-Pos Board B148

AFM-Enhanced Single-Molecule Spectroscopy Studies of Intermittent Coherence and Time Bunching Effect of Enzyme Dynamics

Yufan He, Yuanmin Wang, Maolin Lu, **Hong P. Lu**.
Bowling Green State University, Bowling Green, OH, USA.

Enzymatic reactions are traditionally studied at the ensemble level, despite significant static and dynamic inhomogeneities. Subtle conformational changes play a crucial role in protein functions, especially in enzymatic reactions involving complex substrate-enzyme interactions and chemical reactions. We applied AFM-enhanced single-molecule spectroscopy to study the mechanisms and dynamics of enzymatic reactions involved with kinase and lysozyme proteins. Enzymatic reaction turnovers and the associated structure changes of individual protein molecules were observed simultaneously in real-time by single-molecule FRET detections. Our single-molecule spectroscopy measurements of enzymatic conformational dynamics have revealed time bunching effect and intermittent coherence in conformational state change dynamics involving in enzymatic reaction cycles. The coherent conformational state dynamics suggests that the enzymatic catalysis involves a multi-step conformational motion along the coordinates of substrate-enzyme complex formation and product releasing, presenting as an extreme dynamic behavior intrinsically related to the time bunching effect that we have reported previously. Our results support a multiple-conformational state model, being consistent with a complementary conformation selection and induced-fit enzymatic loop-gated conformational change mechanism in substrate-enzyme active complex formation. Our new approach is applicable to a wide range of single-molecule FRET measurements for protein conformational changes under enzymatic reactions.

1379-Pos Board B149

High-Order Correlated Motion in Single-Molecule Human Adenylate Kinase 1

Chien Y. Lin[1], Jung Y. Huang[1], Leu-Wei Lo[2].
[1]Department of Photonics and Institute of Electro-Optical Engineering, National Chiao Tung University, Hsinchu, Taiwan, [2]Division of Medical Engineering Research, National Health Research Institutes, Zhunan, Taiwan.

The growing body of data shows protein motion plays an important role in the catalytic processes of enzymes. Human adenylate kinase 1 (AK1) is the key enzyme in maintaining the cellular energy homeostasis. The conformational change of AK1 involves large-amplitude rearrangements of the enzyme's lid domain. Observing a single molecule can remove the ensemble average, thus allows the exploration of hidden structural heterogeneity. To observe the enzyme actions of AK1 at the single-molecule level, we developed a data-taking scheme allowing us to observe the single-molecule kinetics in long time without the limitation by photobleaching. We labeled the core domain and the rim of AK with two Alexa-532 chromophores. Due to the self quenching from photo-induced electron transfer, the conformation changes of the core domain can be accurately encoded into the fluorescent intensity traces with a spatial sensitivity <1nm. Because Mg2+ ion is known to be an affecter in the energy signaling network in cells, we first investigated the effect of Mg2+ on the catalytic kinetics of AK. By analyzing the photon traces, we found that the binding of Mg2+ ions to arginines and lysines of AK increases the structural heterogeneity, which then couples to the core domain and changes the catalytic function. We further discovered that ATP/AMP substrate binding suppresses the conformation fluctuation of AK and improves the thermal stability of the core domain. The resulting third-order correlation functions of photon traces do not have time-reversal symmetry, indicating single-molecule AK is in a nonequilibrium steady state. We employed hidden Markov model with Baysian inference to verify that the photon traces are originated from transitions among three states (open, mid, close). Substrate binding to AK causes different domains of AK to move in a sequential order and suppresses the thermally-activated random motions.

1380-Pos Board B150

Spectroscopic Studies of Substrate and Ligand Binding in Inducible Nitric Oxide Synthase

Karin Nienhaus[1], Michael Horn[1], G. Ulrich Nienhaus[1,2].
[1]Karlsruhe Institute of Technology, Karlsruhe, Germany, [2]Department of Physics, University of Illinois, Urbana, IL, USA.

Nitric oxide is a small molecule involved in various physiological processes. It serves for instance as a vasorelaxant or neurotransmitter. The three isoforms of nitric oxide synthase (eNOS, nNOS, iNOS) catalyze the formation of NO in a two-step reaction. In a first step arginine is converted to hydroxy-arginine. Subsequently, this intermediate is cleaved to yield citrulline and NO.
In spite of the increasing interest in these proteins in medical research, little is known about the molecular details of the catalytic mechanism. We have used UV/visible and Fourier Transform Infrared / Temperature Derivative Spectroscopy (FTIR/TDS) over a wide range of temperature (4 - 300 K) to observe the interaction between protein (iNOS), ligand and substrate. In these experiments, the physiological ligand O2 has been replaced by CO to observe the ternary complex without steady turnover of arginine. In particular, we have focused on the influence of arginine, the intermediate hydroxy-arginine and cofactor H4B on ligand binding.

1381-Pos Board B151

A General Expression for the Michaelis-Menten Constant

Rolando C. Rossi.
IQUIFIB-Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

It is sometimes taken for granted that a change in the value of the Michaelis-Menten constant, Km, following the mutation of an enzyme's amino acid is due to a modification in the intrinsic affinity of the active site for the substrate, leading to the unproven conclusion that the original amino acid is part of this site. This, as well as other misinterpretations could be avoided if a clear, general expression of Km were available. The following is proposed:
$Km = (k_{-1} \times f_{ES\infty} + k_{cat})/(k_1 \times f_{E0})$,
where k_1 and k_{-1} are respectively the rate constants for binding and dissociation of the substrate, S, k_{cat} is the catalytic constant, $f_{ES\infty}$ is the fraction of enzyme present as the enzyme-substrate complex at $[S] \rightarrow \infty$ and f_{E0} is the fraction of enzyme able to bind the substrate at [S]=0. These quantities $f_{ES\infty}$ and f_{E0} will be respectively less than unity assuming the existence of intermediates other than the bound form *E*S and the free form *E*, respectively. Alternative forms for this expression can be obtained considering that $k_{cat} = k_2' \times f_{ES\infty}$, where k_2' is the net rate constant for the reaction from *E*S to the next intermediate in the forward direction (Cleland, 1975, Biochemistry 14:3220-3224). The expression is model independent since for every model following Michaelis-Menten kinetics the rate constants k_1 and k_{-1} will be present and the quantities $f_{ES\infty}$, f_{E0}, and k_{cat} (or alternatively, k_2') can be defined. Using this expression to analyze models for the Na-ATPase activity of the sodium pump, which displays Michaelis-Menten kinetics, allows to explain for instance why the value of Km for ATP is about the same as that of Kd, the equilibrium dissociation constant, although the substrate is not in rapid equilibrium with its site on the enzyme.
With grants from CONICET and University of Buenos Aires, Argentina.

1382-Pos Board B152

A New Secretory Phospholipase A2 from Glycine Max Soybean: Purification, Characterization and Kinetic Analysis

Maria E. Mariani[1,2], Ricardo Madoery[2], Gerardo Fidelio[1].
[1]Department of Biochemical Sciences-CIQUIBIC, School of Chemical Sciences, National University of Córdoba, Cordoba, Argentina, [2]Department of Biological Fundamentation, School of Agronomic Sciences- UNC, Córdoba, Argentina.

The Phospholipase A_2 superfamily is a broad and growing group of enzymes that catalyze the stereospecific cleavage of the sn-2 acyl ester union of diacyl-glycerophospholipids and liberate 1-acyl-2-lysophospholipids and free fatty acids. In plants, secretory PLA_2s ($sPLA_2$s) mediate a variety of cellular processes, including growth, defense and stress response. On the other hand, the enzymatically produced lysoderivatives are strong bioemulsifyers with numerous applications in food and pharmaceutical industries. The aim of the present work was to obtain a Phospholipase A_2 enzyme from soybean (*Glycine max*) and study its enzymatic properties. This is the first time a secretory Phospholipase A_2 ($sPLA_2$) from soybean seeds, denoted as $GmsPLA_2$-I, was produced by heterologous expression in *E.coli*, renatured from inclusion bodies by guanidine treatment and purified by ion exchange chromatography. The cDNA encoded a mature protein of 114 amino acid residues with a signal peptide of 24 residues. The amino acid sequence for the mature $GmsPLA_2$-I contains 12 cysteines, the Ca^{2+} binding loop (YGKYCGxxxxGC) and the active site motif (DACCxxHDxC), that are commonly conserved in $sPLA_2$s from plants. Phospholipase A_2 activity was evaluated by means of two techniques to obtain the optimum conditions for catalysis. First, mixed micelles of phospholipid/Triton X-100 were used in a titration assay in order to study the effect of pH and the Ca^{2+} ion on $sPLA_2$ activity and to determine the kinetic parameters (V_{max} and K_m). On the other hand, Langmuir-monolayer assays were performed in order to study the relation of the activity of $GmPLA_2$-I with the